Appendix 13.5-F Nitrite SPO Recommendation Memo

TELKWA COAL LIMITED

Derivation of a Site Performance Objective (SPO) for Nitrite for the Tenas Project

Final Report

October 6, 2021

Submitted to:

Allegiance Coal Limited
Vancouver, BC

Attention: Dan Farmer, Chief Operating Officer

TABLE OF CONTENTS

Page

TABLE OF CONTENTS

Page

List of Tables

List of Figures

TABLE OF CONTENTS

List of Appendices

SIGNATURE PAGE

Josh Baker, M.Sc., P.Chem.
Environmental Chemist
Nautilus Environmental

Guy Gilron, M.Sc., R.P.Bio., ICD.D
Senior Environmental Scientist,
Borealis Environmental Consulting

David DeForest,
Senior Environmental Toxicologist,
Windward Environmental

This report has been prepared by Nautilus Environmental Company Inc., with input and senior review from Windward Environmental and Borealis Environmental, based on data and/or samples provided by our client and the results of this study are for their sole benefit. Any reliance on the data by a third party is at the sole and exclusive risk of that party. The results presented herein relate only to the samples tested.

1.0 BACKGROUND AND INTRODUCTION

In support of the Environmental Assessment for Allegiance Coal's Tenas Metallurgical Coal Project (the Project), Nautilus Environmental Company Inc. (Nautilus) proposed and conducted a battery of toxicity tests utilizing freshwater aquatic species to establish sublethal toxicity thresholds for nitrite (NO_2) (Nautilus Environmental 2021). Geochemical model predictions (conducted by SRK Consulting) have identified potential exceedances of the provincial long-term chronic aquatic life guideline (ALG) for nitrite downstream of the proposed effluent discharge point at the Project. The results of the sublethal toxicity tests are utilized herein to derive appropriate site performance objectives (SPOs).

As outlined in this report, additional information on the sublethal sensitivity of aquatic organisms is available since the establishment of the provincial NO_2 guideline in 1986 (BCMoE, 1986). For example, the long-term ramifications of increased methemoglobin in the blood of salmonids as a result of nitrite exposure, which was the basis of the 1986 ALG (BC MoE, 1986), have been further explored and defined. Furthermore, the sensitivity of aquatic organisms to nitrite, other than salmonids, were not explicitly included in the 1986 ALG derivation. Despite additional information on the toxicity of nitrite to aquatic organisms since 1986, there is a lack of data available pertaining to the sublethal toxicity of nitrite to aquatic life in the vicinity of the Project. Due to the risk of potential nitrite exceedances, additional sublethal toxicity testing with nitrite was rationalized.

Results of sublethal toxicity tests with nitrite conducted on two salmonids, an aquatic macrophyte, an alga, a mayfly, a benthic invertebrate and a pelagic invertebrate have been reported previously (Nautilus Environmental 2021); these data are used herein to derive a chronic environmental benchmark for the Project. The tests used species which are resident to, or downstream of, the Project, or in some cases, closely-related surrogates. Formulated site water ('Tenas water') was used for testing, which matched water chemistry conditions predicted for the Project site, ensuring that the results of the nitrite exposures were specific to the site. Inclusion of representative salmonid, aquatic insect and crustacean species, previously shown to be sensitive to acute nitrite exposure, in the sublethal toxicity testing program, ensures that the derived environmental benchmark will be protective of aquatic life in the vicinity of the mine. The testing conducted contributes significantly to the available data on the sublethal sensitivity of freshwater organisms to nitrite.

BC MOECCS (2019) has recently incorporated the Species Sensitivity Distribution (SSD) approach, which incorporates data from a broad multi-species approach, in the establishment of environmental benchmarks. The SSD approach was applied to the sublethal toxicity nitrite data to derive an environmental benchmark that is protective of resident aquatic life. Specifically, an HC5, representing the Hazardous Concentration that has the potential to affect 5% of the species, was determined from the SSD model. Multiple SSDs, and their resulting HC5s, are presented in this derivation report as an exercise to determine the influence of different species (*i.e.*, site-specific species versus all species) and different effect levels (*i.e.*, EC10, EC20, etc.) on the outcome of the SSDs. Discussion is provided regarding the SSD and HC5 selection, as well as establishment of the long-term (i.e., chronic) environmental benchmark for nitrite.

2.0 REVIEW OF SUBLETHAL TOXICITY OF NITRITE

A review of available information on the sub-chronic or chronic toxicity of nitrite is presented below. First, a review of the studies used to derive the 1986 ALG (BC MoE, 1986) is provided and new information in relation to these studies is presented. Second, a review of available chronic toxicity data for nitrite from the peer-reviewed literature is presented. Finally, the effect concentrations for nitrite derived from the site-specific testing (Nautilus Environmental 2021) are summarized.

2.1 1986 Aquatic Life Guideline

In 1986, the BC Ministry of Environment (BC MoE) established and published a 30-day average guideline of 0.02 mg/L for nitrite as nitrogen (NO_2-N) for the protection of aquatic life (*i.e.*, the long-term [chronic] ALG). The ALG was primarily established based on the results of nitrite exposures with salmonids that demonstrated increased generation of blood methemoglobin (MetHb) which is the form of hemoglobin that is not able to carry oxygen, due to nitrite exposure. Work by Russo and others (Russo and Thurston, 1977; Smith and Russo, 1975), identified that 8-day exposures to concentrations of >0.1 mg/L NO_2-N led to increases in MetHb in rainbow trout. This was similar to the findings of Brown and McLeay (1975), who also reported a significant increase in MetHb in rainbow trout exposed to 0.015 mg/L NO_2-N for 4 days. The BC MoE used these findings, as well as observation of gill alterations in steelhead trout following 4 weeks of exposure to 0.06 mg/L NO_2-N (Wedemeyer and Yasutake, 1976), to establish the resulting 30-day average ALG of 0.02 mg/ L NO_2-N (BC MoE, 1986).

The increased proportion of hemoglobin that was present as MetHb in the salmonid studies used to derive the guideline was not associated with reduced performance of the salmonids (i.e., no reduction in specific growth rate or survival was observed). Statistically-significant ($p < 0.05$) increases in %MetHb in comparison to the controls were observed in the NO_2 exposures by Smith and Russo (1975) and Wedemeyer and Yasutake (1976), with increases from 3.6 to 14.3% and from 1-2 to 3-4%, respectively. However, these NO_2-exposed salmonids were still within a range of %MetHb observed in other unexposed salmonids; %MetHb was found to range from 2 to 17% in unexposed salmonids (Smith and Russo, 1975). Both the Smith and Russo (1975) and Wedemeyer and Yasutake (1976) studies did not result in reduced growth of salmonids. Furthermore, the Brown and McLeay (1975) study observed increases in MetHb up to 70% following nitrite exposure without any observed mortalities.

Studies published since establishment of the current provincial ALG (BC MoE, 1986) have reported that the %MetHb increases that occur in salmonids during the first days of nitrite exposure (*i.e.*, 0-14 days) are subsequently followed by decreases in %MetHb (Doblander and Lackner, 1997; Zuskova et al., 2013). Other studies published since 1986 have reported that salmonids activate coping mechanisms which reverse the initial increases in MetHb of nitrite exposures (Gutierrez et al., 2011), possibly by oxidizing nitrite to nitrate and reducing MetHb back to functional hemoglobin (Ha et al., 2019). These new studies indicate that the initial increases in %MetHb in salmonids, observed within the first days of nitrite exposure in studies used in the 1986 ALG derivation, may be temporary and not associated with any long-term reduced performance of salmonids. Indeed, the Wedemeyer and Yasutake (1976) study, which was used to support the BC MoE ALG derivation, demonstrated that the gill alterations observed in steelhead trout after 3 weeks were absent at 7 and 28 weeks, resulting in no difference in sublethal measures between the nitrite-exposed and non-exposed (control) steelhead trout at the end of the 28-week test exposure period.

There appears to be a lack of a definitive connection between long-term adverse effects to salmonids manifesting from the increases in MetHb, thus implying that this blood parameter may be an inaccurate tool to assess salmonid impairment from nitrite exposure. In agreement with the lack of long-term effects from MetHb, and the likelihood of internal metabolic mechanisms of MetHb, is the low acute-to-chronic ratio (ACR) for nitrite established in the scientific literature (Lewis and Morris, 1986). Therefore, the rationale for the 1986 ALG appears to be established without an association with ecological impairment, and, for the above reasons, studies were excluded from the SSDs conducted within this report in cases where the study observed an increase in MetHb in a manner that was not clearly associated with effects on long-term growth and/or survival of salmonids.

The 1986 ALG derivation identified chloride as a particularly strong toxicity-modifying factor (TMF) for nitrite and, therefore, both acute and chronic guidelines were established as chloride-dependent (BC MoE, 1986). At the time of the ALG derivation, little was known about the relationship between chloride concentration and the chronic toxicity of nitrite. The chronic ALG was derived using a 100:1 ratio of chloride:nitrite, which was suggested to be appropriate for long-term protection, while a 30:1 ratio was used to derive the short-term acute ALG at different chloride concentrations. Since 1986, substantial new data are available on the relationship between toxicity of nitrite and chloride concentration. For example, strong relationships have been reported between acute nitrite effect concentrations and chloride for amphipods, planarians, fish and crayfish (Kroupova et al. 2018), although relationships between chronic toxicity of nitrite and chloride concentrations are not widely reported in the literature. For this reason, chronic toxicity tests with nitrite need to be conducted at chloride concentrations that are representative of the environment being assessed in order to be relevant.

2.2 Available sublethal toxicity data for nitrite

Available sublethal toxicity data for nitrite was obtained using Google and Google Scholar to search for peer-reviewed literature on nitrite toxicity to aquatic organisms. In addition, a targeted search of published papers in the journal Environmental Toxicology and Chemistry was conducted. Key words used for the literature search included: aquatic toxicity, nitrite, salmonids, invertebrates, aquatic plants, algae, sublethal and chronic. Sublethal data were compiled from chronic exposures (*i.e.,* occurring during a relatively long-term period representing a significant portion of the life span of the organism, such as 10% or more) and subchronic exposures (from sensitive sublethal tests used as short-term methods to estimate chronic toxicity). Acute data (*i.e.,* < generally 7-d in duration) were not compiled as part of the literature search. Studies were included if they contained the appropriate information regarding the nitrite exposure and could meet the criteria for consideration as primary or secondary data, consistent with BC MOECCS (2019).

Sixteen studies from the literature were considered appropriate; these included eight studies with amphibians, two with invertebrates, five with fish and a single study with a cyanobacterium. Effect concentrations available included 10, 20 and 50% effect levels (*i.e.,* EC10, EC20 and EC50), no and lowest observed effect concentrations (*i.e.*, NOEC and LOEC) and maximum acceptable toxicant concentrations (MATC). Studies which were excluded are described in Section 2.2.1.

2.2.1 Excluded and revised data

Some of the studies that were reviewed were excluded from the data summary due to the fact that the species studied did not occur in North America. This included studies with catfish, eels, prawns, zebrafish and rohu carp. One mesocosm study was excluded due to the presence of other nitrogen species at elevated concentrations. Other studies were removed from consideration due to insufficient data or poor relationships between the nitrite exposure and the adverse effect reported. This included the following studies, which were excluded for the following reasons:

- Shinn et al., 2013: A non-monotonic dose response was observed;
- Griffis-Kyle, 2007: no statistical analysis was provided to define adverse effects;
- Voslarova et al., 2008: no analysis of nitrite concentrations were reported and high background nitrate was present in the control and dilution water; and,
- Wuertz et al., 2013: histological performance did not appear to relate to the nitrite treatments.

In reviewing the two Kroupova studies (*i.e.*, Kroupova et al., 2008; Kroupova et al., 2010), discrepancies were noted between the authors' establishment of NOEC and LOEC values and the data presented. In both studies, the NOEC and LOECs were reported as the LC0 and LC10 values, respectively. These LC0 and LC10 values were much lower than concentrations at which statistically reduced growth of the rainbow trout and common carp, respectively. Therefore MATCs were calculated from concentrations demonstrated to statistically reduce growth (LOEC) and the adjacent concentration which demonstrated no statistically-significant adverse effect (NOEC).

Table 1. Sublethal toxicity of nitrite to freshwater organisms (available in the scientific literature).

Reference	Taxon	Species	Life stage	Duration	Endpoint	EC10	EC20	EC50	LOEC	MATC
						mg/L NO_2-N				
Marco and Blaustein 1999	amphibian	*Rana cascadae*	Gosner 39-40	14-d	development				3.5	
Marco et al. 1999	amphibian	*Rana pretiosa*	7 dph	15-d	survival			0.57		
Marco et al. 1999	amphibian	*Rana aurora*	7 dph	15-d	survival			1.19		
Marco et al. 1999	amphibian	*Bufo boreas*	7 dph	15-d	survival			1.75		
Marco et al. 1999	amphibian	*Hyal regilla*	7 dph	15-d	survival			1.23		
Marco et al. 1999	amphibian	*Ambystoma gracile*	7 dph	15-d	survival			1.01		
Griffis-Kyle 2005	amphibian	*Ambystoma tigrinum*	larval	26-d	survival				4.6	
Griffis-Kyle 2005	amphibian	*Rana sylvatica*	larval	34-d	survival				0.5	
Chen et al. 2011	cyanobacteria	*Microcystis aeruginosa*	NA	15-d	growth				5.0	
Dave and Nilsson 2005	invertebrate	*Ceriodaphnia dubia*	<24h	8-d	reproduction				0.22	
US EPA 2010	invertebrate	*Ceriodaphnia dubia*	<24h	8-d	reproduction	1.4	1.7	2.4		1.2

Table 1. continued

US EPA 2010	fish	*Pimephales promelas*	embryo-larval	32-d	growth	9.3	11.0	14.6		10.1
Adelman et al. 2009	fish	*Pimephales promelas*	embryo-larval	32-d	growth and survival					2.96
Adelman et al. 2009	fish	*Notropis topeka*	juvenile	30-d	survival					4.96
Kroupova et al. 2008	fish	*Oncorhynchus mykiss*	juvenile	28-d	growth				3.0	1.7
Kroupova et al. 2010	fish	*Cyprinus carpio*	embryo-larval	29-d	survival				28	

EC: Effect concentration, LOEC: Lowest Observed Effect Concentration, MATC: Maximum Acceptable Toxicant Concentration, dph: days post hatch, NA: Not applicable

2.3 Site-specific sublethal toxicity data for nitrite

Water chemistry can have a significant influence on nitrite aquatic toxicity. Increases in chloride, pH, alkalinity, dissolved oxygen, bromide, hardness and humic substances have all been demonstrated to reduce nitrite toxicity (Kroupova et al., 2005; Kroupova et al., 2018; Meinelt et al., 2010). Chloride is a particularly strong modifying factor of acute nitrite toxicity to freshwater organisms (Russo et al., 1981; Alonso and Camargo, 2008; Kroupova et al., 2018). The importance of TMFs, particularly chloride, on nitrite toxicity increases the importance of conducting nitrite toxicity tests incorporating water chemistry conditions that are relevant to the Project site. Water chemistry conditions in the Project area are characterized by low chloride (*i.e.*, <2 mg/L), and high bicarbonate and hardness (*i.e.,* 150 mg/L as $CaCO_3$, respectively) (ERM, 2019).

Results of acute rainbow trout tests conducted in formulated Tenas site water agreed with established relationships of nitrite toxicity under differing chloride conditions derived from the literature (Nautilus Environmental 2021). Therefore, the site-specific sublethal data derived in formulated Tenas water, and presented herein, are the most representative data to be applied to assess the possibility of adverse effects due to nitrite exposure for the Project.

The results of the site-specific sublethal toxicity testing conducted as part of this study (Nautilus Environmental 2021) are summarized in Table 2. Of the six sublethal toxicity tests conducted, no significant reduction in performance was observed in three and, therefore, only NOEC endpoints were generated for those tests. The other three tests had significant adverse effects resulting in a variety of effects endpoints.

2.3.1 Lowest effect concentrations

The Lowest Effect Concentrations (or LECx) were calculated based on the method described in de Bruyn and Elphick (2013), where x represents the percent minimum significant difference(PMSD) from each test (Dunnett's Comparison test). Briefly, the non-linear regression or linear interpolation model of the sublethal data is used to calculate the nitrite concentration at which a PMSD level of effect is observed. For example, if an 11% effect on biomass is the effect level that can be statistically detected as significant from the control organism response, the nitrite concentration necessary to cause this level of inhibition is calculated (*i.e.,* LEC11 or IC11) from the dose-response. In the *C. dubia*, *N. triangulifer* and *O. mykiss* sublethal tests, PMSDs were determined as 16.4, 36.6 and 10.4%, respectively, and therefore LECx endpoints presented in Table 2 represent IC16, IC37 and IC10, respectively.

The BC MOECCS guidance for the derivation of Water Quality Guideline for Aquatic Life (Section 4; BC MOECCS 2019) states that an EC/IC representing a no-effects threshold is the most preferred endpoint over more ubiquitously-applied effect levels, such as the EC10 or EC20. As the LECx represents the exact point at which a no-effect becomes a statistically significant effect, it therefore reflects the most preferred endpoint based on the BC MOECCS criteria. The LECx methodology also allows for use of regression-based statistical data evaluation, which BC MOECCS also prefers.

2.3.2 Application of Acute-to-Chronic ratio for toxicity endpoints of salmonids (rainbow trout and coho salmon)

Two uncertainties were associated with the salmonid toxicity data generated in the sublethal toxicity testing study (Nautilus Environmental 2021). First, the study determined that nitrite toxicity differed depending upon the life stage of salmonids used in the toxicity tests; a significant increase in sensitivity to nitrite was observed in juvenile rainbow trout in comparison to larval rainbow trout. Because the less-sensitive life stage (*i.e.,* larval) was used for the sublethal exposures with rainbow trout, the potential for increased sensitivity of larger rainbow trout needed to be addressed prior to inclusion in the SSD analysis. Second, acute toxicity effect concentrations (*i.e*., 96-h LC50s) were determined for coho salmon, while no sublethal toxicity information was generated, due to logistical limitations (*e.g*., lack of available coho salmon of an appropriate life stage).

To address these two uncertainties, an ACR for salmonids was generated from the acute and chronic tests conducted with rainbow trout. A salmonid ACR of 3.9 was calculated by dividing the 96-h survival LC50 (*i.e.,* 2.75 mg/L) by the 14-d biomass inhibition concentrations from nitrite exposures in site water with larval rainbow trout. This ACR was then applied to the 96-h nitrite LC50s for juvenile rainbow trout and juvenile coho salmon (*i.e.,* 1.16 mg/L and 1.14 mg/L, respectively), to establish estimates of a sublethal effect thresholds, for the more sensitive life stage.

Table 2. Summary of sublethal toxicity endpoints for nitrite to freshwater organisms tested in formulated Tenas site water (Nautilus Environmental, 2021).

Taxon	Species	Life stage	Duration	Endpoint	EC10	EC20	EC50	NOEC	LECx
					mg/L NO_2-N				
Sublethal									
Fish	*Oncorhynchus mykiss*	larval	14-d	biomass	0.71	1.03	>1.25	-	0.72
invertebrate	*Chironomus dilutus*	3rd instar	10-d	growth	>1.28	>1.28	>1.28	1.28	
invertebrate	*Ceriodaphnia dubia*	<24h	6-d	reproduction	0.10	0.15	0.32	-	0.136
invertebrate	*Neocloeon triangulifer*	<24h	14-d	biomass	0.039	0.046	0.069	-	0.059
aquatic plant	*Lemna minor*	NA	7-d	growth	>1.29	>1.29	>1.29	1.29	
aquatic plant	*Pseudokirchneriella subcapitata*	NA	72-h	growth	>1.37	>1.37	>1.37	1.37	
Acute									
fish	*Oncorhynchus kisutch*	juvenile	96-h	survival			1.14		
fish	*Oncorhynchus mykiss*	juvenile	96-h	survival			1.16		

EC: Effect concentration, NOEC: No Observed Effect Concentration, LECx: Lowest Effect Concentration, NA: Not applicable

3.0 SPECIES SENSITIVITY DISTRIBUTIONS

Species Sensitivity Distributions (SSDs) represent the variation in sensitivity of species to a chemical parameter by a statistical or empirical distribution function of responses for a sample of species (Posthuma et al., 2002). The statistical distribution is then used to calculate a concentration expected to be safe to most species of interest. The approach employed by both the BC MOECCS and Canadian Council of Ministers of the Environment (CCME, 2007) has been to use the Hazardous Concentration affecting 5% of the species (HC5) from the SSD to establish water quality guidelines and objectives.

3.1 Methodology

The *ssdtools shiny web* application was used to estimate HC5 values (BC MOECCS, 2021). This website uses the R package (ssdtools version 0.2.0) to plot the toxicity data using maximum likelihood estimation (Thorley and Schwarz, 2018) (MLE). SSDs were developed for a variety of scenarios to assess the influencing factors affecting the calculated HC5. Three distributions, (*i.e.,* log-logistic, log-normal and gamma) were fitted to the toxicity data from each scenario and a model averaging approach was taken to estimate an HC5 value, in each case. These scenarios were initially conducted to help inform which scenario would be appropriate for use in the SPO.

Scenarios 1, 2 and 3 were SSDs developed using only site-specific data (*i.e.*, Section 2.3) and using differing endpoints (*i.e.,* EC10s, EC20s and LECxs, respectively). Scenarios 4, 5 and 6 were SSDs that were calculated using site-specific data with external literature values (*i.e.*, Section 2.2) and using differing endpoints (*i.e.,* EC10s, EC20s and LECxs, respectively). An SSD was calculated for an additional scenario (Scenario 7), that included the site-specific data and literature values from studies with chloride concentrations that were consistent with the Project site conditions (*i.e.,* 0.5-5.0 mg/L Cl). Values for fathead minnow and Topeka shiner tests with relevant chloride concentrations were available from Adelman et al. (2009) and for a *C. dubia* test from US EPA (2010) under these chloride concentrations. Because *C. dubia* was one of the test species for the site-specific testing, the geometric mean of chronic values was calculated for inclusion in the SSD. Other data from the literature that did not report chloride concentrations or with reported higher chloride concentrations (*i.e.*, >5.0 mg/L Cl) were excluded in this case. As the majority of literature values, and three of the site-specific data, were hypothesis-test-based results (*i.e.,* NOEC, LOEC and MATC) the values used in the SSD from these tests did not change when different endpoint scenarios (*i.e.*, EC10, EC20, LECx) were used. The sublethal salmonid endpoints derived in the site-specific study were adjusted according to the uncertainties addressed in Section 2.3.2.

Following the choice of which data set scenario to employ for the SPO, a more detailed inspection of all the SSD models (*i.e.,* log-normal, gamma, Weibull, log-logistic, log-gumbel) was conducted to ensure that statistically-appropriate models were included in the derivation of the HC5. The best models were selected based on goodness-of-fit tests, as well as a visual assessment of the lower tails of the distribution where the HC5 occurs. The results of this analysis are included with the presentation of the SPO in Section 4 and in Appendix A.

3.2 Species Sensitivity Distribution Results

The results generated for the various SSD scenarios are presented in Table 3; SSD models for site-specific data are illustrated in Figures 1 through 3 and for the combination of site-specific and literature values in Figures 4 through 7. The SSD analyses resulted in HC5s ranging from 0.0348 to 0.127 mg/L NO_2-N across the various scenarios. When literature values were included in the SSDs, the resulting HC5 increased two to three-fold compared to SSDs that only included site-specific data. This was particularly important for the location of the HC5 relative to the sensitive data points, as inclusion of the literature values, which were generally less sensitive than the site-specific values, resulted in the HC5 falling between the lowest (*N. triangulifer*) and second lowest (*C. dubia*) values. Utilization of the LECx endpoint, which was available for four of the species, resulted in an HC5 that was intermediate to HC5s generated when EC10s and EC20s were used.

Table 3. Summary of Hazard Concentrations (HC5) and variance estimates for nitrite generated from Species Sensitivity Distributions.

Scenario/Figure	Description	Endpoints used	HC5[1] (mg/L NO_2-N)	SE	LCL	UCL
1	Internal Site-specific EC10s	EC10	**0.0348**	0.0717	0.00628	0.271
2	Internal Site-specific EC20s	EC20	**0.0545**	0.0837	0.0106	0.317
3	Internal Site-specific LECx	LECx	**0.0504**	0.0758	0.0106	0.290
4	Internal Site-specific and external EC10s	EC10	**0.103**	0.0654	0.0442	0.292
5	Internal Site-specific and external EC20s	EC20	**0.127**	0.0686	0.0583	0.318
6	Internal Site-specific LECx and external MATCs	LECx	**0.122**	0.0735	0.0555	0.348
7	Internal Site-specific and external EC10/MATCs with site chloride	EC10	**0.0553**	0.0977	0.0094	0.355

SE: Standard Error, LCL: Lower Confidence Limit, UCL: Upper Confidence Limit, EC: Effect concentration, MATC: Maximum Acceptable Toxicant Concentration, LECx: Lowest Effect Concentration

1- Each HC5 was calculated using the model-average of log-logistic, log-normal and gamma using the *ssdtools shiny web application*

Figure 1. Species Sensitivity Distribution using EC10s from site-specific sublethal toxicity testing with nitrite [HC5: 0.0348 mg/L NO_2-N] .

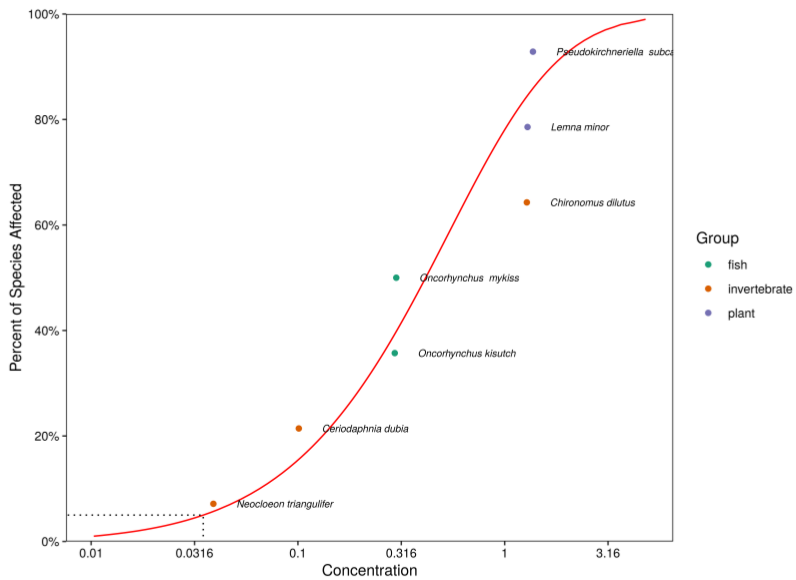


Figure 2. Species Sensitivity Distribution using EC20s from site-specific sublethal toxicity testing with nitrite [HC5: 0.0545 mg/L NO_2-N].

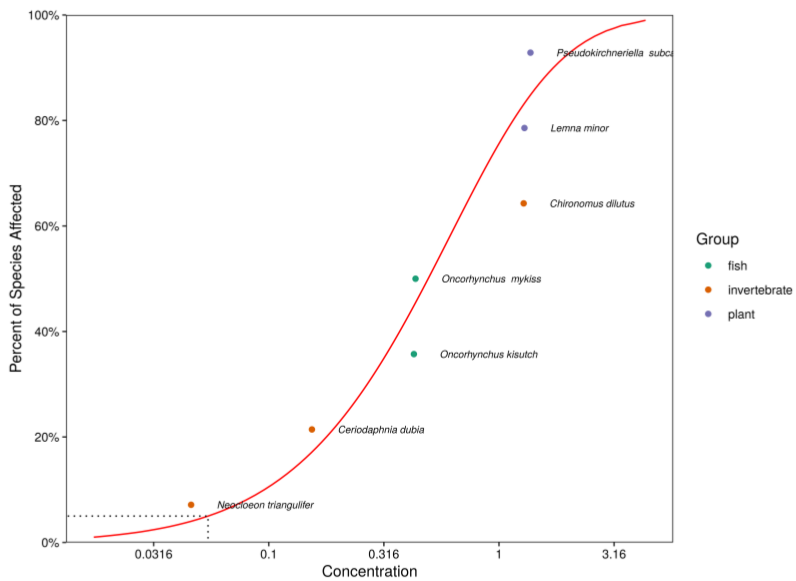


Figure 3. **Species Sensitivity Distribution using LECxs from site-specific sublethal toxicity testing with nitrite [HC5: 0.0504 mg/L NO_2-N].**

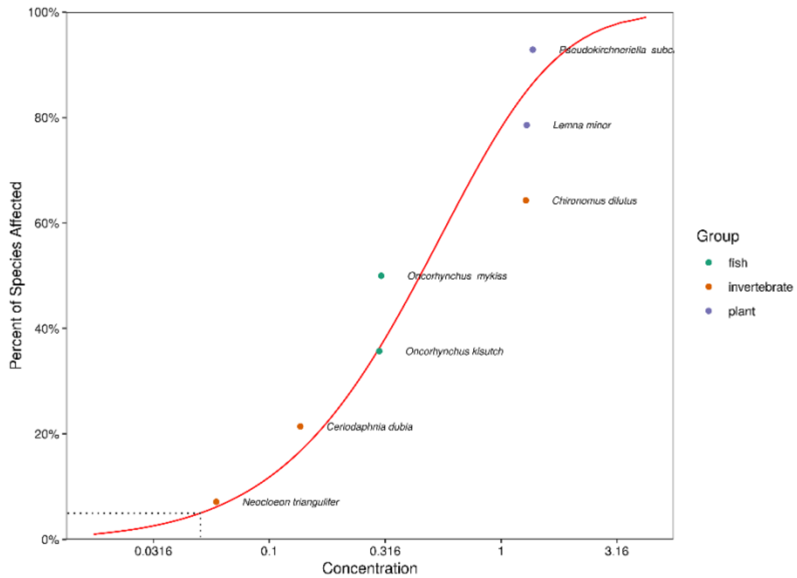


Figure 4. **Species Sensitivity Distribution using EC10s for nitrite from site-specific sublethal toxicity testing and all peer-reviewed literature regardless of chloride condition [HC5: 0.103 mg/L NO_2-N].**

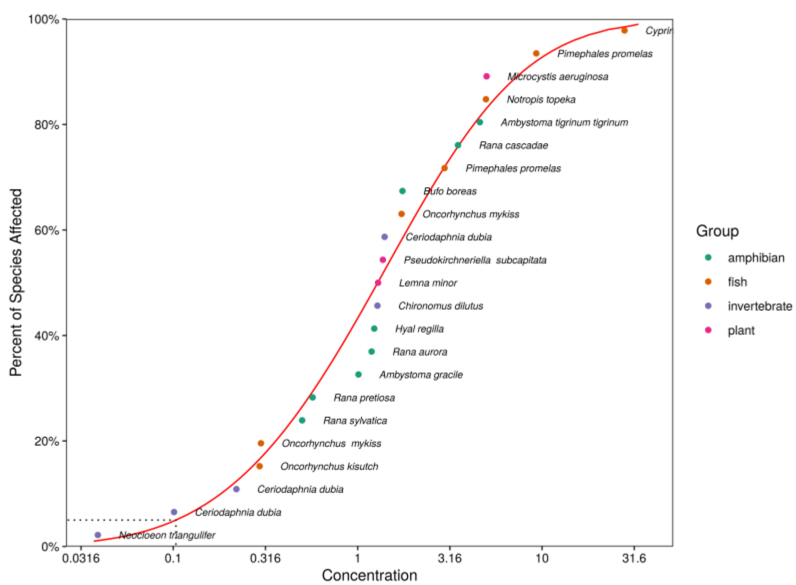


Figure 5. Species Sensitivity Distribution using EC20s for nitrite from site-specific sublethal toxicity testing and all peer-reviewed literature regardless of chloride condition [HC5: 0.127 mg/L NO_2-N].

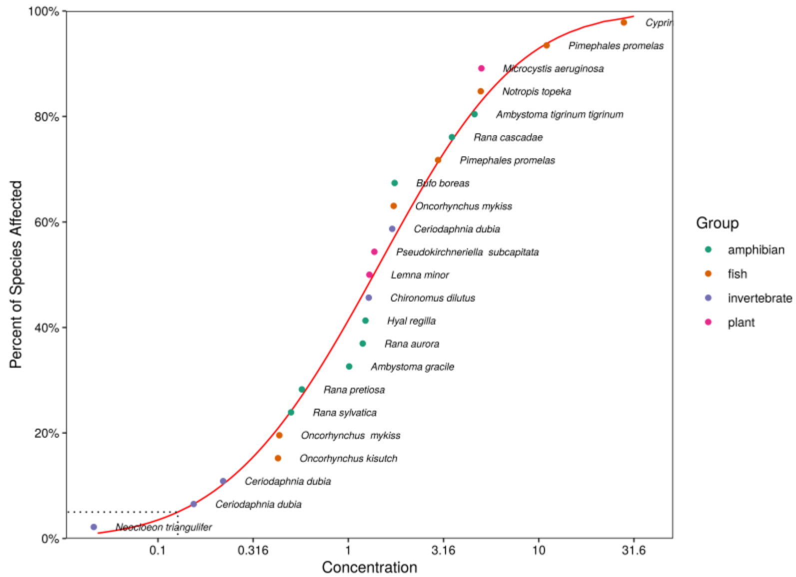


Figure 6. Species Sensitivity Distribution using LECxs for nitrite from site-specific sublethal toxicity testing and all peer-reviewed literature regardless of chloride condition [HC5: 0.0.122 mg/L NO_2-N].

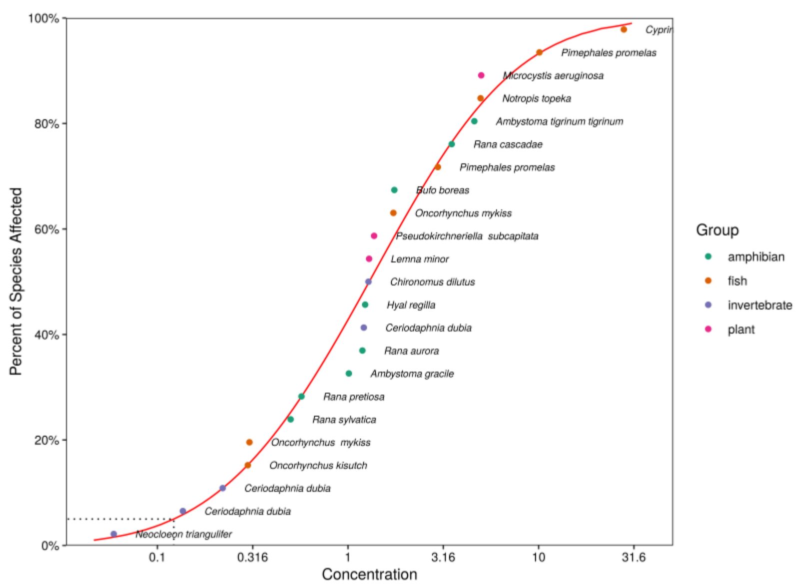
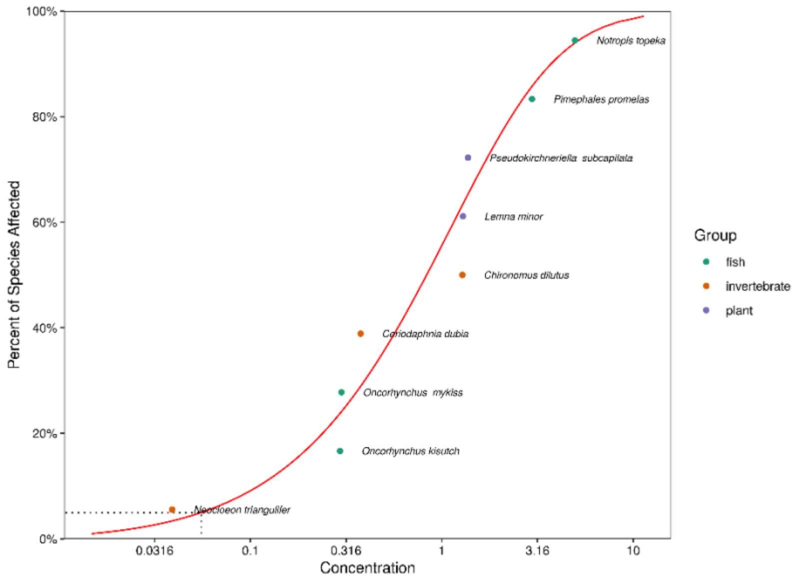


Figure 7. Species Sensitivity Distribution using EC10s for nitrite from site-specific sublethal toxicity testing and peer-reviewed literature with ambient chloride of 0.5-5.0 mg/L [HC5: 0.0553 mg/L NO_2-N].



4.0 SITE PERFORMANCE OBJECTIVE FOR THE TENAS PROJECT

The information generated from calculating SSDs with various data set combinations was used to ensure that the SPO for nitrite is protective of aquatic organisms downstream of the Tenas project. Scenarios with literature values (*i.e.,* not based on site-specific toxicity tests) included in SSDs were conducted, because these published data may include a sensitive species that was not tested as part of the site-specific toxicity test battery (*e.g.,* amphibians). Inclusion of literature values (Scenarios 4, 5 and 6), resulted in a higher HC5, in comparison to site-specific data alone, and an HC5 that was substantially greater than the endpoint for the most sensitive species (*i.e.,* the mayfly, *N. triangulifer*). An HC5 that is substantially greater than the lowest endpoint could result in an inadequate level of protection for this sensitive species and, therefore, SSDs that included all external literature values were removed from consideration for the SPO. In addition, it was not known if the majority of literature values were less sensitive than the site-specific HC5 values due to a lower sensitivity of the species tested, or if it was due to the conditions under which the species was tested, specifically, the chloride concentration. Therefore, it was considered more appropriate to include literature values derived from exposures that had chloride concentrations that were consistent with Project site conditions.

The scenario that included the site-specific EC10s and literature values from tests conducted under relevant chloride concentrations was determined to be the most appropriate data set for developing the SPO; therefore, an SSD was calculated for this scenario using a more detailed approach. A closer inspection of the SSD models derived with this data set determined that three models (*i.e.,* gamma, log-normal and Weibull) fit the data well (*e.g.,* delta AICs < 1; Appendix A) and, therefore, all three were used in a three-model-average to derive the HC5. The HC5 that resulted from this model was 0.052 mg/L NO_2-N (Table 4, Figure 8). This value is proposed as the SPO for nitrite downstream of the Tenas project.

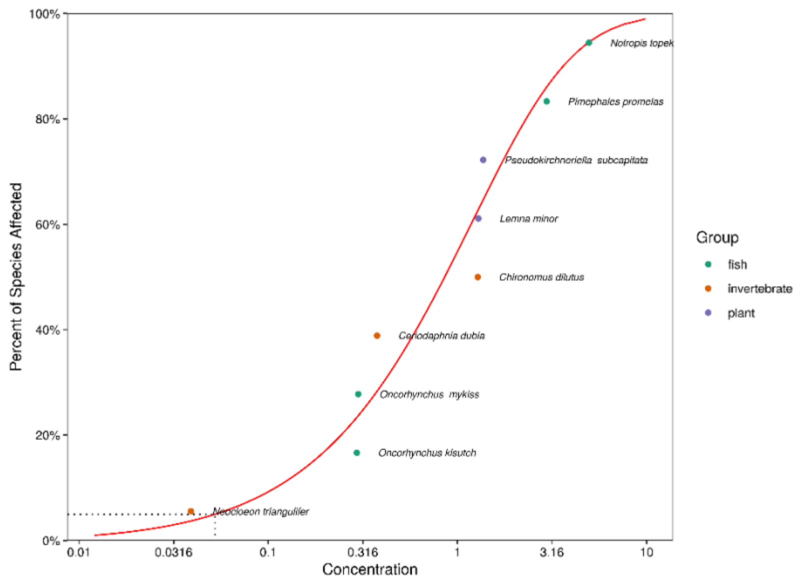
The proposed SPO uses EC10 values, rather than EC20s or LECx values, which provides a higher level of conservatism than the other scenarios. This is of particular importance for the mayfly, which was 2 to 3 times more sensitive to nitrite than any of the other species tested. Moreover, the proposed SPO is below the lowest nitrite concentration determined to significantly adversely affect the mayfly (*i.e.*, the LECx for biomass of 0.059 mg/L NO_2-N; determined from the 14-d exposure). Therefore, the proposed SPO would be expected to provide protection for this species as well as the other resident species.

Table 4. Hazard Concentration (HC5), and proposed Site Performance Objective, for nitrite derived using Species Sensitivity Distributions.

Description	Model	HC5 and SPO (mg/L NO_2-N)	SE	LCL	UCL
Site-specific EC10s and published EC10/MATCs with site chloride	Model average of gamma, log-normal and Weibull	**0.0524**	0.103	0.0096	0.381

SE: Standard Error, LCL: Lower Confidence Limit, UCL: Upper Confidence Limit

Figure 8. Species Sensitivity Distribution (three-model-average with gamma, log-normal and Weibull) using EC10s for nitrite incorporating data from site-specific sublethal toxicity testing and chloride-appropriate peer-reviewed literature endpoints [HC5: 0.0524 mg/L NO_2-N].



5.0 REFERENCES

Adelman IR, Kusilek LI, Koehle J, Hess J. 2009. Acute and chronic toxicity of ammonia, nitrite, and nitrate to the endangered Topeka shiner (*Notropis topeka*) and fathead minnows (*Pimephales promelas*). *Environmental Toxicology and Chemistry*, 28(10), 2216-2223.

Almendras JME. 1987. Acute nitrite toxicity and methemoglobinemia in juvenile milkfish (*Chanos chanos Forsskal*). *Aquaculture*, 61(1), 33–40.

Alonso A, Camargo JA. 2008. Ameliorating effect of chloride on nitrite toxicity to freshwater invertebrates with different physiology: A comparative study between amphipods and planarians. *Archives of Environmental Contamination and Toxicology*, 54(2), 259–265.

British Columbia Ministry of Environment and Parks. 1986. Water quality criteria for nitrogen (nitrate, nitrite and ammonia). Water Quality Unit, Victoria, BC. Prepared by R.N Nordin and L.W. Pommen. November 1986.

British Columbia Ministry of Environment and Climate Change Strategy. 2019. Derivation of water quality guidelines for the protection of aquatic life in British Columbia. Water Quality Guideline Series, WQG-06. Prov. B.C., Victoria B.C.

British Columbia Ministry of Environment and Climate Change Strategy. 2021. ssd tools shiny app accessed on March 29, 2021. https://bcgov-env.shinyapps.io/ssdtools/.

Brown DA, McLeay DJ. 1975. Effect of Nitrite on Methemoglobin and Total Hemoglobin of Juvenile Rainbow Trout. *The Progressive Fish-Culturist*, 37(1), 36–38.

CCME. 2007. A protocol for the derivation of water quality guidelines for the protection of aquatic life 2007. Canadian Environmental Quality Guidelines: Canadian Council of Ministers of the Environment (CCME).

Chen W, Liu H, Zhang Q, Dai S. 2011. Effect of nitrite on growth and microcystins production of *Microcystis aeruginosa* PCC7806. *Journal of Applied Phycology*., 23, 665-671.

Dave G, Nilsson E. 2005. Increased reproductive toxicity of landfill leachate after degradation was caused by nitrite. *Aquatic Toxicology*, 73(1), 11–30.

deBruyn AMH and Elphick JR. 2013. Proposing a vigorous hybrid of the LOEC and ECx. *Integrated Environmental Assessment and Management*. 9, 345-347.

Doblander C, Lackner R. 1997. Oxidation of nitrite to nitrate in isolated erythrocytes: a possible mechanism for adaptation to environmental nitrite. *Canadian Journal of Fisheries and Aquatic Sciences,* 54, 157–161.

ERM. 2019. Tenas Project: 2017 to 2019 Baseline Report. Prepared for Telkwa Coal Limited. May 2019; Project #0403488.

Griffis-Kyle KL. 2005. Ontogenic delays in effects of nitrite exposure on tiger salamanders (*Ambystoma tigrinum tigrinum*) and wood frogs (*Rana sylvatica*). *Environmental Toxicology and Chemistry*, 24(6), 1523-1527.

Griffis-Kyle KL. 2007. Sublethal effects of nitrite on eastern tiger salamander (*Ambystoma tigrinum tigrinum*) and wood frog (*Rana sylvatica*) embryos and larvae: implications for field populations. *Aquatic Ecology,* 41, 119-127.

Gutierrez A, Kolarevic J, Sæther B, Bæverfjord G, Takle H, Medina H, Terjesen B. 2011. Effects of sub-lethal nitrite exposure at high chloride background during the parr stage of Atlantic salmon. *In: Aquaculture Europe 2011 Proc.,* Rhodes, Greece. October, 1080–1081.

Ha NTK, Huong DTT, Phuong NT, Bayley M, Jensen FB. 2019. Impact and tissue metabolism of nitrite at two acclimation temperatures in striped catfish (*Pangasianodon hypophthalmus*). *Aquatic Toxicology*, *212*, 154–161.

Kroupova H, Machova J, Svobodova Z. 2005. Nitrite influence on fish: A review. *Veterinarni Medicina*, 50(11), 461–471.

Kroupova H, Machova J, Piackova V, Blahova J, Dobsikova R, Novotny L, Svobodova Z. 2008. Effects of subchronic nitrite exposure on rainbow trout (*Oncorhynchus mykiss*). *Ecotoxicology and Environmental Safety*, 71(3), 813–820.

Kroupova H, Prokes M, Macova S, Penaz M, Barus V, Novotny L, Machova J. 2010. Effect of nitrite on early-life stages of common carp (*Cyprinus carpio L.*). *Environmental Toxicology and Chemistry*, 29(3), 535–540.

Kroupová H, Valentová O, Svobodová Z, Šauer P, Máchová J. 2018. Toxic effects of nitrite on freshwater organisms: a review. *Reviews in Aquaculture*, 10(3), 525–542.

Marco A and Blaustein AR. 1999. The effects of nitrite on behaviour and metamorphosis in cascades frogs (*Rana cascadae*). *Environmental Toxicology and Chemistry*, 18(5), 946-949.

Marco A, Quilchano C, Blaustein AR. 1999. Sensitivity to nitrate and nitrite in pond-breeding amphibians from the Pacific Northwest, USA. *Environmental Toxicology and Chemistry*, 18(12), 2836-2839.

Meinelt T, Kroupova H, Stüber A, Rennert B, Wienke A, Steinberg CEW. 2010. Can dissolved aquatic humic substances reduce the toxicity of ammonia and nitrite in recirculating aquaculture systems? *Aquaculture*, 306(1–4), 378–383.

Nautilus Environmental. 2021. Sublethal toxicity testing of nitrite: Tenas Project, Telkwa, BC. Final report submitted to Allegiance Coal Limited (Vancouver, BC). January 12, 2021. WO# 201781-787 and 202083.

Posthuma L, Suter GW, Traas TP. 2002. Species sensitivity distributions in ecotoxicology. Lewis Publishers, Boca Raton, FL, USA.

Russo CR, Smith, C. E., & Thurson, R. V. 1974. Acute Toxicity of Nitrite to Rainbow Trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada*. 31(10), 1653–1655.

Russo, CR, Smith C and RV Thurston. 1981. Acute Toxicity of Nitrite to Rainbow Trout (*Salmo gairdneri*): Effects of pH, Nitrite species, and Anion Species. *Canadian Journal of Fisheries and Aquatic Sciences,* 38, 387-393.

Russo RC, Thurston RV. 1977. The acute toxicity of nitrite to fishes. In: Tubb RA (ed.) Recent Advances in Fish Toxicology: A Symposium, pp. 118–131. United States Environmental Protection Agency, Corvallis.

Shinn C, Marco A, Serrano L. 2013. Influence of low levels of water salinity on toxicity of nitrite to anuran larvae. *Chemosphere*, 92(9), 1154-1160.

Smith CE, Russo RC. 1975. Nitrite-Induced Methemoglobinemia in Rainbow Trout. *The Progressive Fish-Culturist*, 37(3), 150–152.

US EPA. 2010. Final Report on Acute and Chronic Toxicity of Nitrate, Nitrite, Boron, Manganese, Fluoride, Chloride and Sulfate to Several Aquatic Animal Species. United States Environmental Protection Agency, Office of Science and Technology Health and Ecological Criteria Division and Region 5 Water Division. EPA 905-R-10-002.

Voslarova E, Pistekova V, Svobodova Z, Bedanova I. 2008. Nitrite toxicity to *Danio rerio*: Effects of subchronic exposure on fish growth. *Acta Veterinaria Brno*, 77, 455-460.

Wedemeyer GA, Yasutake WT. 1978. Prevention and Treatment of Nitrite Toxicity in Juvenile Steelhead Trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada*, 35(6), 822–827.

Wuertz S, Schulze SGE, Eberhardt U, Schulz C, Schroeder JP. 2013. Acute and chronic nitrite toxicity in juvenile pike-perch (*Sander lucioperca*) and its compensation by chloride. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*, 157(4), 352–360.

Zusková E, Máchová J, Velíšek J, Stará A, Svobodová Z, Kroupová HK. 2013. Recovery of rainbow trout (*Oncorhynchus mykiss*) after subchronic nitrite exposure. *Acta Veterinaria Brno*, 82(1), 73–79.

APPENDIX A – SSD information

END OF REPORT